

SUPPLEMENTARY MATERIALS

**Genetic events in the progression of adenoid cystic carcinomas of the breast to high-grade
triple-negative breast cancer**

Fusco et al.

Supplementary Tables 1-5

Supplementary Figure 1

Supplementary Table 1: List of antibodies, clones, dilutions, and antigen retrieval methods.

Marker	Clone	Dilution	Antigen retrieval	Company
ER	EP1	RTU	Heat-induced epitope retrieval (HIER), Dako PT Link, 20'	DAKO
PR	PgR636	RTU	EnVision FLEX Target Retrieval Solution, high pH, 20'	DAKO
HER2 (HercepTest)	A0485	RTU	HercepTest Epitope Retrieval Solution, 40'	DAKO
Ki67	MIB1	1:400	ER2, 30'	DAKO
CK7	OV-TL 12/30	1:1600	CC1, 24'	DAKO
p63	4A4	RTU	CC1, 24'	Ventana
c-Kit	pAb	1:500	CC1,32'	DAKO

CC1, Cell Condition 1 Solution, Ventana, Roche; RTU, ready-to-use.

Supplementary Table 2: Primers used for *MYB-NFIB* transcript detection by RT-PCR.

Primer set	<i>MYB</i> primer (exon)/ sequence	<i>NFIB</i> primer (exon)/sequence	Exons tested
1	1910 F: (14) 5'-AGCTCCGTTTTAATGGCACC-3'	1096R (8c): 5'-GGGTATAAATGCCTGCCGTT-3'	14-8c
2	1925 F: (14) 5'-GCACCAGCATCAGAAGATGA-3'	1197R (9): 5'-CCGTAAGATGGGTGTCCTA-3'	14-9
3	1925 F (14): 5'-GCACCAGCATCAGAAGATGA-3'	862R (8a): 5'-GCCAGGCACTTCCCTACTAC-3'	14-8a
4	1693 F (12): 5'-GCAGGATGTGATCAAACAGG-3'	1197R (9): 5'-CCGTAAGATGGGTGTCCTA-3'	12-9
5	1432 F (10): 5'-CTCAGACTTGAAATGCCTTC-3'	1197R (9): 5'-CCGTAAGATGGGTGTCCTA-3'	10-9
6	1334 F (9): 5'-CAGGGCACCATTCTGGATAA-3'	1952R (9): 5'-GTGCTGCAATTGCTGGTCTA-3'	9-9
7	1334 F (9): 5'-CAGGGCACCATTCTGGATAA-3'	1197R (9): 5'-CCGTAAGATGGGTGTCCTA-3'	9-9

Primers as previously described by Fehr et al, J Pathol 2011 and Martelotto et al, J Pathol 2015.

Supplementary Table 3: List of the 254 genes and 341 genes included in the customized breast cancer and MSK-IMPACT targeted massively parallel sequencing assays, respectively.

	Breast panel	MSK-IMPACT		Breast panel	MSK-IMPACT		Breast panel	MSK-IMPACT		Breast panel	MSK-IMPACT
1	ABCA13	ABL1	101	GPS2	ESR1	201	RAD51C	MUTYH	301		SMARCB1
2	ABCB1	AKT1	102	GRB2	ETV1	202	RAD51D	MYC	302		SMARCD1
3	ADAMTSL1	AKT2	103	GRIN2A	ETV6	203	RAD52	MYCL1	303		SMO
4	AGFG2	AKT3	104	GRIN2B	EZH2	204	RAD54B	MYCN	304		SOCS1
5	AHNAK2	ALK	105	HECW1	FAM123B	205	RAD54L	MYD88	305		SOX17
6	AK9	ALOX12B	106	HERC2	FAM175A	206	RAF1	MYOD1	306		SOX2
7	AKAP9	APC	107	HIF1A	FAM46C	207	RB1	NBN	307		SOX9
8	AKT1	AR	108	HIST1H3B	FANCA	208	RBBP8	NCOR1	308		SPEN
9	AKT2	ARAF	109	HRAS	FANCC	209	RELN	NF1	309		SPOP
10	AKT3	ARID1A	110	HRNR	FAT1	210	RICTOR	NF2	310		SRC
11	ANK3	ARID1B	111	HSP90AA1	FBXW7	211	RIF1	NFE2L2	311		STAG2
12	AOAH	ARID2	112	HSP90AB1	FGF19	212	RPGR	NKX2-1	312		STK11
13	APC	ARID5B	113	HUWE1	FGF3	213	RPS6KB1	NKX3-1	313		STK40
14	APOBEC1	ASXL1	114	IGF1R	FGF4	214	RPTOR	NOTCH1	314		SUFU
15	APOBEC2	ASXL2	115	INPP4B	FGFR1	215	RUNX1	NOTCH2	315		SUZ12
16	APOBEC3A	ATM	116	IRS1	FGFR2	216	SAAL1	NOTCH3	316		SYK
17	APOBEC3C	ATR	117	JAK1	FGFR3	217	SF3B1	NOTCH4	317		TBX3
18	APOBEC3D	ATRX	118	JAK2	FGFR4	218	SHC1	NPM1	318		TERT
19	APOBEC3F	AURKA	119	KIT	FH	219	SHROOM4	NRAS	319		TET1
20	APOBEC3G	AURKB	120	KMT2C	FLCN	220	SMO	NSD1	320		TET2
21	APOBEC3H	AXIN1	121	KMT2D	FLT1	221	SOS1	NTRK1	321		TGFBR1
22	APOBEC4	AXIN2	122	KRAS	FLT3	222	SPEN	NTRK2	322		TGFBR2
23	ARAF	AXL	123	LAMA1	FLT4	223	SPRY1	NTRK3	323		TMEM127
24	ARID1A	B2M	124	LAMA5	FOXA1	224	SPTA1	PAK1	324		TMPRSS2
25	ATM	BAP1	125	MACF1	FOXL2	225	SRCAP	PAK7	325		TNFIP3
26	ATN1	BARD1	126	MAP1A	FOXP1	226	STAT1	PALB2	326		TNFRSF14
27	ATR	BBC3	127	MAP2K1	FUBP1	227	STAT3	PARK2	327		TOP1
28	ATRX	BCL2	128	MAP2K2	GATA1	228	SVEP1	PARP1	328		TP53
29	AURKA	BCL2L1	129	MAP2K3	GATA2	229	TBL1XR1	PAX5	329		TP63
30	AURKB	BCL2L11	130	MAP2K4	GATA3	230	TBX3	PBRM1	330		TRAF7
31	AURKC	BCL6	131	MAP2K6	GNA11	231	TENM1	PDCD1	331		TSC1
32	BIRC5	BCOR	132	MAP3K1	GNAQ	232	TGFBR1	PDGFRA	332		TSC2
33	BRAF	BLM	133	MAP3K10	GNAS	233	TGFBR2	PDGFRB	333		TSHR
34	BRCA1	BMPR1A	134	MAP3K4	GREM1	234	TGFBR3	PDPK1	334		U2AF1
35	BRCA2	BRAF	135	MAP4K4	GRIN2A	235	TOP2A	PHOX2B	335		VHL
36	BRIP1	BRCA1	136	MAPK1	GSK3B	236	TP53	PIK3C2G	336		VTCN1
37	CACNA1A	BRCA2	137	MAPK8	H3F3C	237	TP53BP1	PIK3C3	337		WT1
38	CACNA1C	BRD4	138	MAPK9	HGF	238	TSC1	PIK3CA	338		XIAP
39	CACNA1E	BRIP1	139	MDM2	HIST1H1C	239	TSC2	PIK3CB	339		XPO1
40	CBFB	BTK	140	MDN1	HIST1H2BD	240	TYK2	PIK3CD	340		YAP1
41	CDC25A	CARD11	141	MED12	HIST1H3B	241	UBR4	PIK3CG	341		YES1
42	CDC25B	CASP8	142	MET	HNF1A	242	USP36	PIK3R1			
43	CDC25C	CBFB	143	MGAM	HRAS	243	WDFY3	PIK3R2			
44	CDH1	CBL	144	MGMT	ICOSLG	244	XBP1	PIK3R3			
45	CDK1	CCND1	145	MLH1	IDH1	245	XPA	PIM1			
46	CDK4	CCND2	146	MLH3	IDH2	246	XPC	PLK2			
47	CDK6	CCND3	147	MRE11A	IFNGR1	247	XRCC1	PMAIP1			
48	CDKN1A	CNE1	148	MSH2	IGF1	248	XRCC2	PMS1			
49	CDKN1B	CD274	149	MSH3	IGF1R	249	XRCC3	PMS2			
50	CDKN2A	CD276	150	MSH5	IGF2	250	ZFHX3	PNRC1			
51	CDKN2B	CD79B	151	MSH6	IKBKE	251	ZFHX4	POLE			
52	CEP164	CDC73	152	MST1L	IKZF1	252	ZFP36L1	PPP2R1A			
53	CHD4	CDH1	153	MTOR	IL10	253	ZNF384	PRDM1			
54	CHD6	CDK12	154	MUTYH	IL7R	254	ZNF703	PRKAR1A			
55	CHEK1	CDK4	155	MXRA5	INPP4A	255		PTCH1			
56	CHEK2	CDK6	156	MYB	INPP4B	256		PTEN			
57	COL12A1	CDK8	157	NBEAL2	INSR	257		PTPN11			
58	CTCF	CDKN1A	158	NBN	IRF4	258		PTPRD			
59	CTNNB1	CDKN1B	159	NBPF1	IRS1	259		PTPRS			
60	CUBN	CDKN2A	160	NCOA3	IRS2	260		PTPRT			
61	DCHS2	CDKN2B	161	NCOR1	JAK1	261		RAC1			
62	DCLRE1C	CDKN2C	162	NCOR2	JAK2	262		RAD50			
63	DEPTOR	CHEK1	163	NEB	JAK3	263		RAD51			
64	DMC1	CHEK2	164	NF1	JUN	264		RAD51B			
65	DOCK11	CIC	165	NF2	KDM5A	265		RAD51C			
66	EGFR	CREBBP	166	NR1H2	KDM5C	266		RAD51D			
67	EIF4A2	CRKL	167	NRAS	KDM6A	267		RAD52			
68	EME1	CRLF2	168	PALB2	KDR	268		RAD54L			
69	EME2	CSF1R	169	PARP1	KEAP1	269		RAF1			
70	EPPK1	CTCF	170	PARP2	KIT	270		RARA			
71	ERBB2	CTLA4	171	PARP3	KLF4	271		RASA1			

72	ERBB3	CTNNB1	172	PAXIP1	KRAS	272		RB1
73	ERBB4	CUL3	173	PCNXL2	LATS1	273		RBM10
74	ERCC1	DAXX	174	PDGFRA	LATS2	274		RECQL4
75	ERCC2	DCUN1D1	175	PDGFRB	LMO1	275		REL
76	ERCC3	DDR2	176	PGR	MAP2K1	276		RET
77	ERCC5	DICER1	177	PIK3CA	MAP2K2	277		RFWD2
78	ESR1	DIS3	178	PIK3CB	MAP2K4	278		RHOA
79	ESR2	DNMT1	179	PIK3R1	MAP3K1	279		RICTOR
80	FAM157B	DNMT3A	180	PLEC	MAP3K13	280		RIT1
81	FANCA	DNMT3B	181	PLK1	MAPK1	281		RNF43
82	FANCB	DOT1L	182	PLXNA4	MAX	282		ROS1
83	FANCC	E2F3	183	PMS1	MCL1	283		RPS6KA4
84	FANCD2	EED	184	PMS2	MDC1	284		RPS6KB2
85	FANCE	EGFL7	185	POLB	MDM2	285		RPTOR
86	FANCF	EGFR	186	POLD1	MDM4	286		RUNX1
87	FANCG	EIF1AX	187	POLE	MED12	287		RYBP
88	FANCI	EP300	188	POLH	MEF2B	288		SDHA
89	FANCL	EPCAM	189	POLQ	MEN1	289		SDHAF2
90	FANCM	EPHA3	190	PRKCA	MET	290		SDHB
91	FBN1	EPHA5	191	PRKCB	MITF	291		SDHC
92	FGFR1	EPHB1	192	PRKCD	MLH1	292		SDHD
93	FGFR2	ERBB2	193	PRKCG	MLL	293		SETD2
94	FGFR3	ERBB3	194	PRKD1	KMT2C	294		SF3B1
95	FGFR4	ERBB4	195	PTCH1	KMT2D	295		SH2D1A
96	FMN2	ERCC2	196	PTEN	MPL	296		SHQ1
97	FOXA1	ERCC3	197	PTK2	MRE11A	297		SMAD2
98	FOXC2	ERCC4	198	RAD50	MSH2	298		SMAD3
99	FRG1B	ERCC5	199	RAD51	MSH6	299		SMAD4
100	GATA3	ERG	200	RAD51B	MTOR	300		SMARCA4

Gray: genes overlapping between the two sequencing panels.

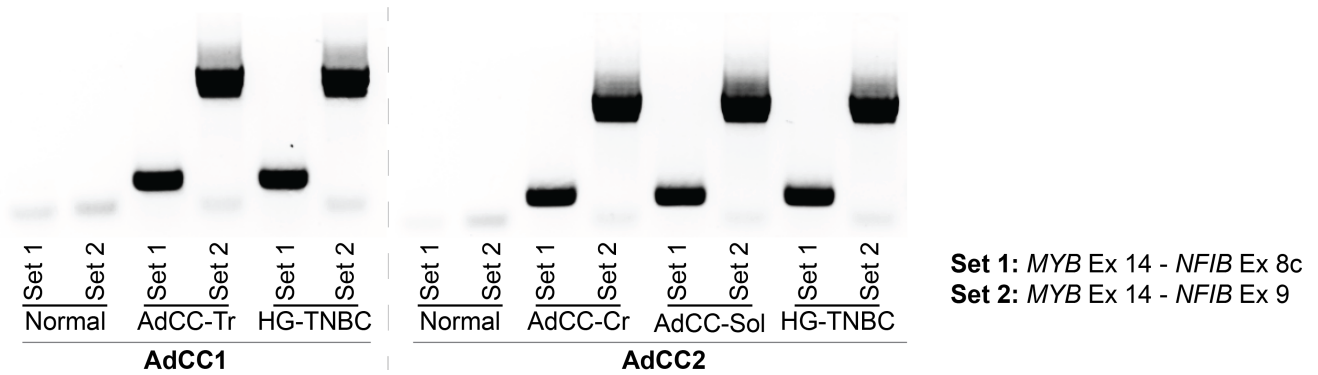
Supplementary Table 4: Primer sets used for validation of mutations using amplicon re-sequencing.

Gene	Forward	Reverse
<i>NOTCH1</i>	GAAGGGGTGCTCAGGCAC	AGCCTCACCTGGTGCAGA
<i>RPS6KB2</i>	AACTGACCGACTTTGGACTCTG	GGTTCTCTGCGGTGAAGG
<i>EP300</i>	ACATGGGGATGGGCACTA	AAGGCAAACCCTCCATCC
<i>MYB</i>	CCTGATCTCAGAGGGTGGAG	GGCTTACATTTTGTGCTTCAGA
<i>KDM6A</i>	GGTGATCGAATGGAGGCTAT	AACTTATTGATGATTTCCATTTCCA
<i>STAG2</i>	GGCAGTTTCTTCTCTGTCCTTT	TATCATAATTAAACATCCTTACCTTCC

Supplementary Table 5. Somatic single nucleotide variants (SNVs) and insertion/deletions (indels) identified in AdCCs and associated HG-TNBCs using massively parallel sequencing.

Sample ID	Gene	Mutation type	Amino acid	Chromosome	Position	Reference allele	Alternative allele	Mutant allele fraction	Sequencing depth	Cancer cell fraction	Sequencing depth amplicon re-sequencing	Clonal/Subclonal mutation	Mutation Taster	CHASM	Kandath et al. Nature 2013	Cancer gene census	Lawrence et al. Nature 2014	Validation
AdCC1-Trab	EP300	STOP_GAINED	R86*	22	41513352	C	T	3.30%	333	20%	7300	Subclonal	A	.	Yes	Yes	Yes	MiSeq
AdCC1-Trab	EP300	STOP_GAINED	R202*	22	41513700	C	T	8.70%	242	53%	7386	Subclonal	A	.	Yes	Yes	Yes	MiSeq
AdCC1-Trab	ERBB2	NON_SYNONYMOUS_CODING	G292R	17	37866707	G	C	1.21%	494	7%	NA	Subclonal	D	Driver	No	Yes	Yes	Cross panel
AdCC1-Trab	FGFR1	NON_SYNONYMOUS_CODING	P314L	8	38282115	G	A	1.0%	394	6%	NA	Subclonal	D	Driver	No	Yes	No	Cross panel
AdCC1-Trab	NOTCH1	FRAME_SHIFT	D2442fs	9	139390864	C	CGT	11.87%	273	74%	2124	Subclonal	.	.	Yes	Yes	Yes	MiSeq
AdCC1-Trab	NOTCH1	FRAME_SHIFT	G2430fs	9	139390901	GC	G	16.10%	264	97%	6827	Clonal	.	.	Yes	Yes	Yes	MiSeq
AdCC1-Trab	RPS6KB2	NON_SYNONYMOUS_CODING	R248Q	11	67200632	G	A	9.00%	299	55%	3311	Subclonal	D	Passenger	No	No	No	MiSeq
AdCC1-HG-TNBC	EP300	STOP_GAINED	R202*	22	41513700	C	T	31.80%	130	97%	6145	Clonal	A	.	Yes	Yes	Yes	MiSeq
AdCC1-HG-TNBC	ERBB2	NON_SYNONYMOUS_CODING	G292R	17	37866707	G	C	4.80%	312	16%	NA	Subclonal	D	Driver	No	Yes	Yes	Cross panel
AdCC1-HG-TNBC	FGFR1	NON_SYNONYMOUS_CODING	P314L	8	38282115	G	A	14.30%	231	48%	NA	Subclonal	D	Driver	No	Yes	No	Cross panel
AdCC1-HG-TNBC	NOTCH1	FRAME_SHIFT	D2442fs	9	139390864	C	CGT	29.49%	286	100%	7150	Clonal	.	.	Yes	Yes	Yes	MiSeq
AdCC1-HG-TNBC	RPS6KB2	NON_SYNONYMOUS_CODING	R248Q	11	67200632	G	A	10.90%	303	37%	2854	Subclonal	D	Passenger	No	No	No	MiSeq
AdCC2-Crib	KMT2C	NON_SYNONYMOUS_CODING	E3717K	7	151859513	C	T	27.35%	245	100%	NA	Clonal	D	Passenger	Yes	Yes	Yes	Cross panel
AdCC2-Crib	KMT2C	NON_SYNONYMOUS_CODING	K339N	7	151962290	C	G	2.78%	144	12%	NA	Subclonal	D	Passenger	Yes	Yes	Yes	Cross panel
AdCC2-Sol	KMT2C	NON_SYNONYMOUS_CODING	E3717K	7	151859513	C	T	30.00%	170	100%	NA	Clonal	D	Passenger	Yes	Yes	Yes	Cross panel
AdCC2-Sol	KMT2C	NON_SYNONYMOUS_CODING	K339N	7	151962290	C	G	5.56%	90	40%	NA	Subclonal	D	Passenger	Yes	Yes	Yes	Cross panel
AdCC2-Sol	MYB	NON_SYNONYMOUS_CODING	R190H	6	135513503	G	A	4.98% (MiSeq)	1099 (MiSeq)	38% (MiSeq)	1099	Subclonal	D	Passenger	No	Yes	No	MiSeq
AdCC2-HG-TNBC	KMT2C	NON_SYNONYMOUS_CODING	E3717K	7	151859513	C	T	27.95%	161	100%	NA	Clonal	D	Passenger	Yes	Yes	Yes	Cross panel
AdCC2-HG-TNBC	KMT2C	NON_SYNONYMOUS_CODING	K339N	7	151962290	C	G	8.22%	73	35%	NA	Subclonal	D	Passenger	Yes	Yes	Yes	Cross panel
AdCC2-HG-TNBC	MYB	NON_SYNONYMOUS_CODING	R190H	6	135513503	G	A	8.55%	234	45%	865	Subclonal	D	Passenger	No	Yes	No	MiSeq
AdCC2-HG-TNBC	STAG2	NON_SYNONYMOUS_CODING	L480F	X	123195095	C	T	14.89%	94	49%	7335	Subclonal	D	Passenger	Yes	Yes	Yes	MiSeq
AdCC2-HG-TNBC	KDM6A	FRAME_SHIFT	G100fs	X	44820600	AG	A	12.90%	155	42%	7694	Subclonal	.	.	Yes	Yes	Yes	MiSeq
AdCC2-HG-TNBC	CDK12	STOP_GAINED	Q1216*	17	37682455	C	T	6.87%	131	36%	1416	Subclonal	.	.	Yes	Yes	Yes	MiSeq

Supplementary Figure 1



Supplementary Figure 1: Results of RT-PCR analysis assessing the presence and type of the *MYB-NFIB* fusion transcript. In all components of AdCC1 and AdCC2 the *MYB-NFIB* fusion transcript consisted of *MYB* exon 14 fused to *NFIB* exon 8c. AdCC-Cr, cribriform adenoid cystic carcinoma; AdCC-Sol, solid adenoid cystic carcinoma; AdCC-Tr, trabecular adenoid cystic carcinoma; Ex, exon; HG-TNBC, high-grade triple-negative breast cancer.